



## Acquiring MRI brain images in awake unrestrained sheep

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## Introduction

Magnetic Resonance Imaging (MRI) is a non-invasive technique that requires the participant to be completely motionless. To date, MRI in awake and unrestrained animals has only been achieved with humans and dogs (Andics et al., 2014). For other species, alternative techniques such as anesthesia (Barrière et al., 2019), restraint (Stefanacci et al., 1998) and/or sedation (Behroozi et al., 2018) have been necessary. MRI studies with sheep have only been conducted under general anesthesia. This ensures the absence of movement and allows relatively long MRI experiments. However, it can also be detrimental to health, disrupt neurovascular coupling (Aksenov et al., 2015) and does not permit the study of higher-level cognition. Here we present a proof of concept that positive reinforcement training in sheep is sufficient to be able to acquire MRI images of their brain. Specifically, we are currently using functional MRI to study the mechanisms of vocalization perception in these farm animals.

## Method

### 1. Step-by-step training protocol with 10 lambs (5 ♂ – 5 ♀)

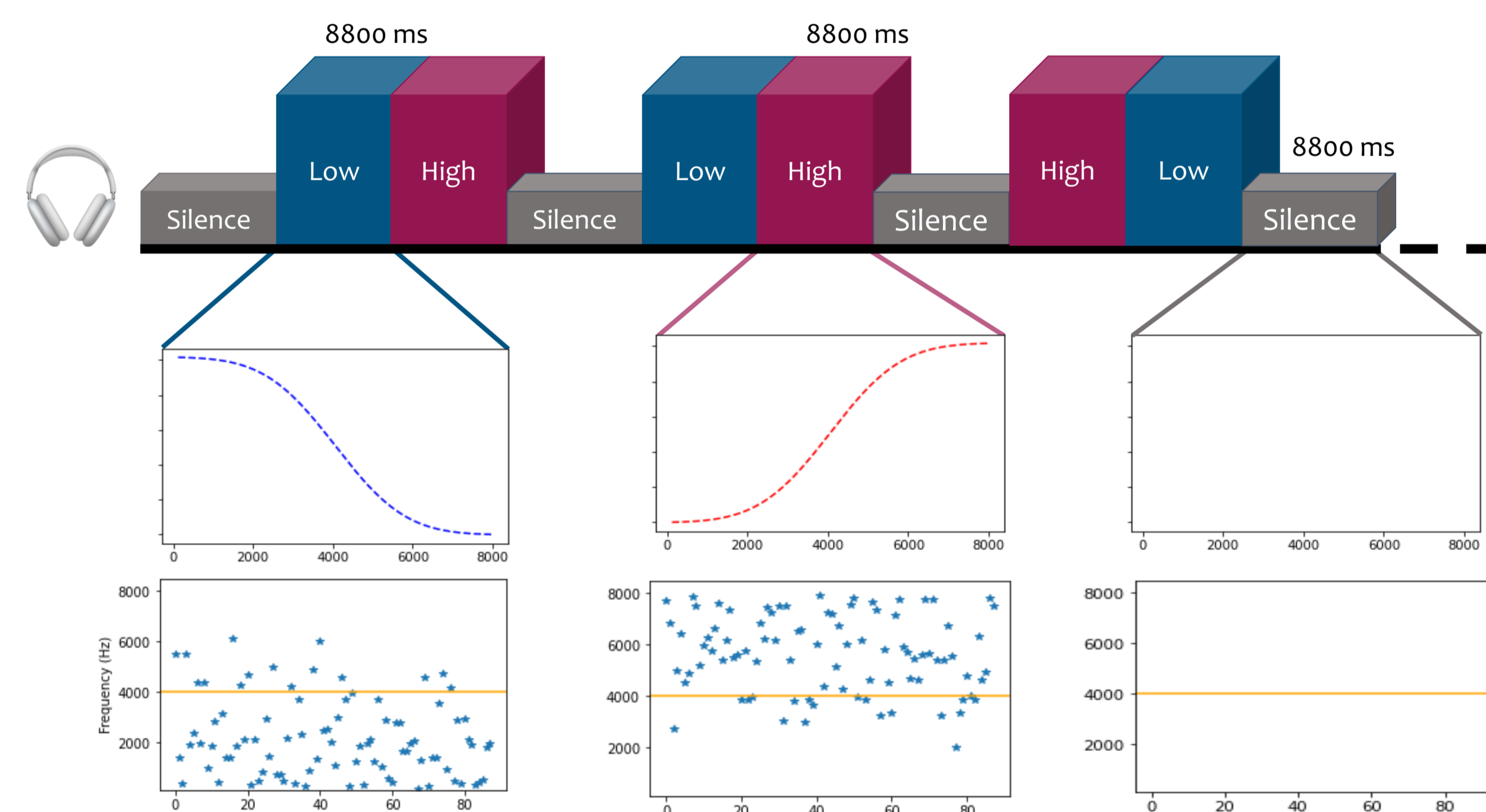
→ Lying motionless on the MRI table for the minimum time required for an acquisition (~10 min)

- **Sheepfold training phase**  
(mock MRI table and coil, scanner noise desensitization)
- **MRI room training phase**  
(table movements, scanner bore, MRI coil, headphones)

### 2. MRI acquisition

→ Localize the sheep auditory cortex and identify its functional organization

#### ■ Auditory stimulation paradigm



#### ■ MRI sequences

**T1-weighted structural scan:** TR = 2200 ms, TE = 2.64 ms, flip angle = 12°, voxel size = 1.12 mm<sup>3</sup>, TA = 2 min 36 sec

**Gradient echo fieldmap:** TR = 500 ms, TE = 4.92/7.38 ms, flip angle=55°, voxel size = 1.5 x 1.5 x 2 mm, TA = 1 min 57 sec

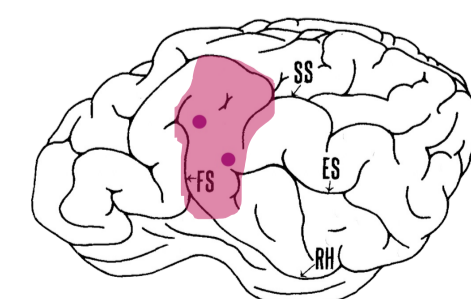
**EPI functional images:** multiband acceleration factor = 2, TR = 2000 ms, TE = 40 ms, flip angle = 12°, number of slices = 40, voxel size = 1.51 mm<sup>3</sup>, number of volumes = 112, TA = 4 min 04 sec

## Discussion

❖ Positive reinforcement training of sheep and positive human – animal cooperation are sufficient to enable acquiring MRI images of their brain.

❖ This innovative training protocol opens the door to the possibility of conducting animal welfare friendly functional MRI studies with sheep.

❖ The preliminary awake sheep fMRI results localize the auditory cortex similarly to the putative region proposed by Michaloudi and colleagues.



(Michaloudi et al., 1986)

## Acknowledgements

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# Sheep voluntarily participate in awake and unrestrained MRI sessions after positive reinforcement-based training



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## ACQUIRING MRI BRAIN IMAGES IN AWAKE UNRESTRAINED SHEEP

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LAMB'S  
BIRTH  
March 2022



### GROUP SELECTION

- ❖ Male and female lamb's under artificial feeding
- ❖ Positive interactions with the lambs twice a day, 7 days a week
- ❖ New objects / Exercises [foam, ramp]

March 2022



### SHEEPFOLD TRAINING PHASE

- ❖ Climb a ramp to reach the mock MRI
- ❖ Put their head inside the mock MRI coil
- ❖ Lie still on a moving table

March 2022 to September 2022



### MRI ROOM TRAINING PHASE

- ❖ Accept headphones on their ears
- ❖ Put their head inside the real MRI coil
- ❖ Lie still on the moving table
- ❖ Stay motionless inside the scanner bore

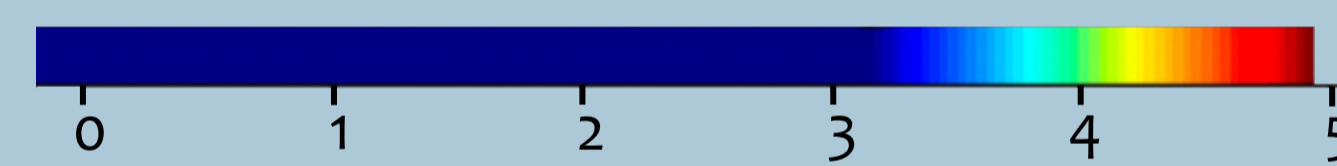
September 2022 to now

	Robin	Brook	Maggie	Ted	Léonard	Tony	Lily	Joe	Jackson	Barnita
Lie down on the table	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Wear headphones	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Head inside the coil	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Scanner bore	✓	✓	✓	✓	✓	✓	✓	✓		✓
Stay motionless (~10 min)	✓	✓	✓	✓	✓					

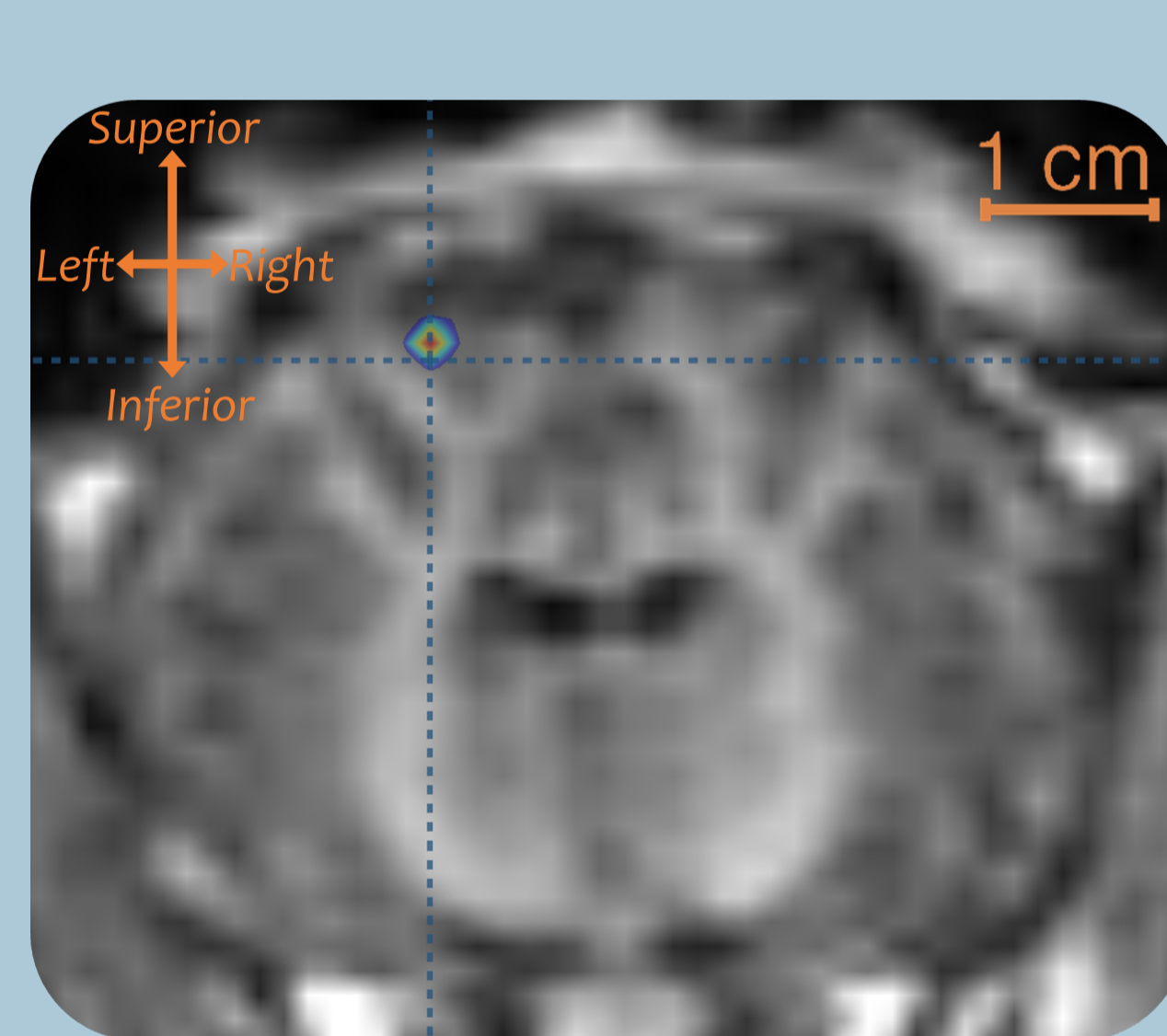
Training Progress. The different steps performed in the MRI room for each sheep  
♀ : Robin, Brook, Maggie, Lily, Barnita ; ♂ : Ted, Léonard, Tony, Joe, Jackson



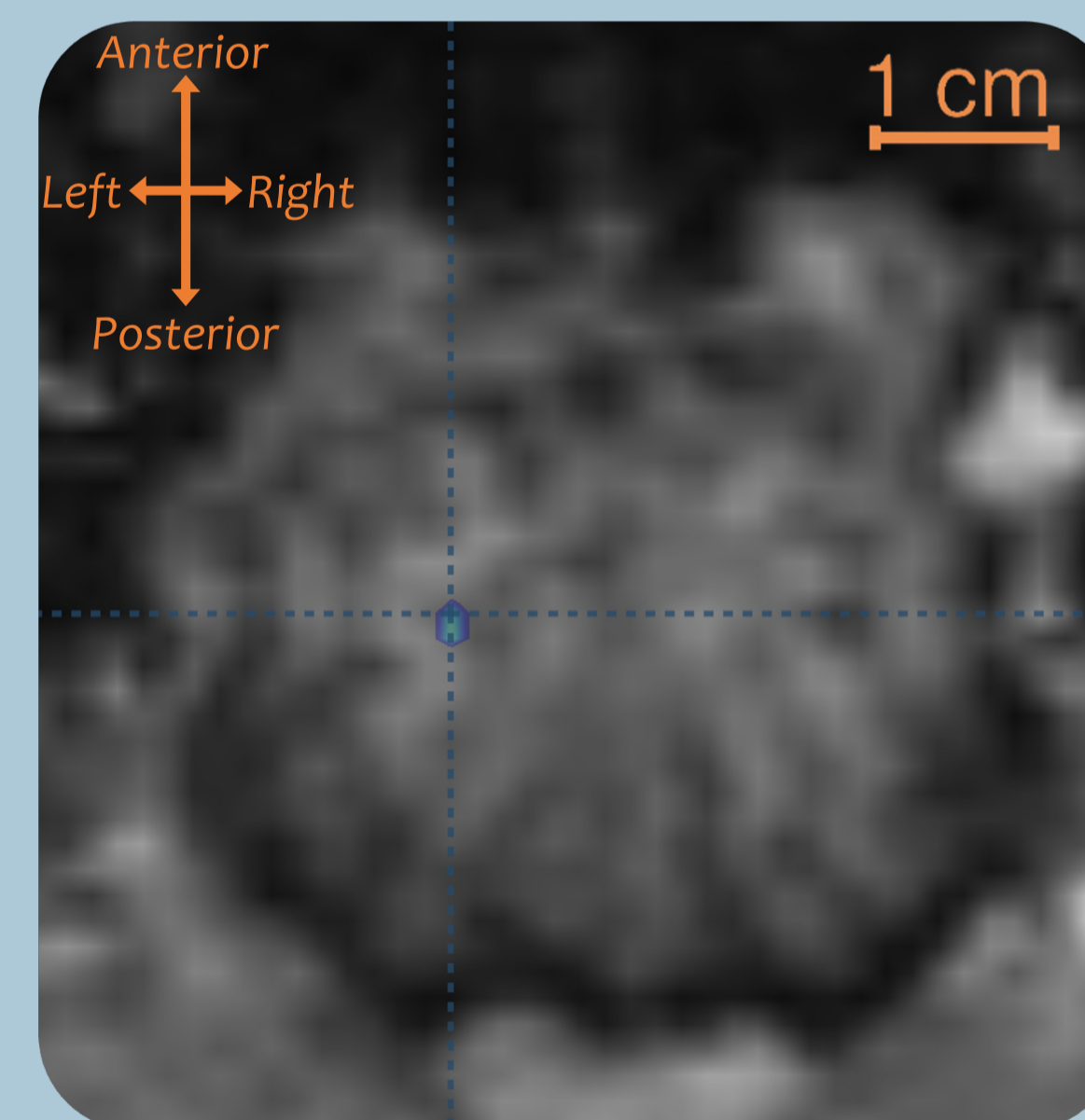
LÉONARD



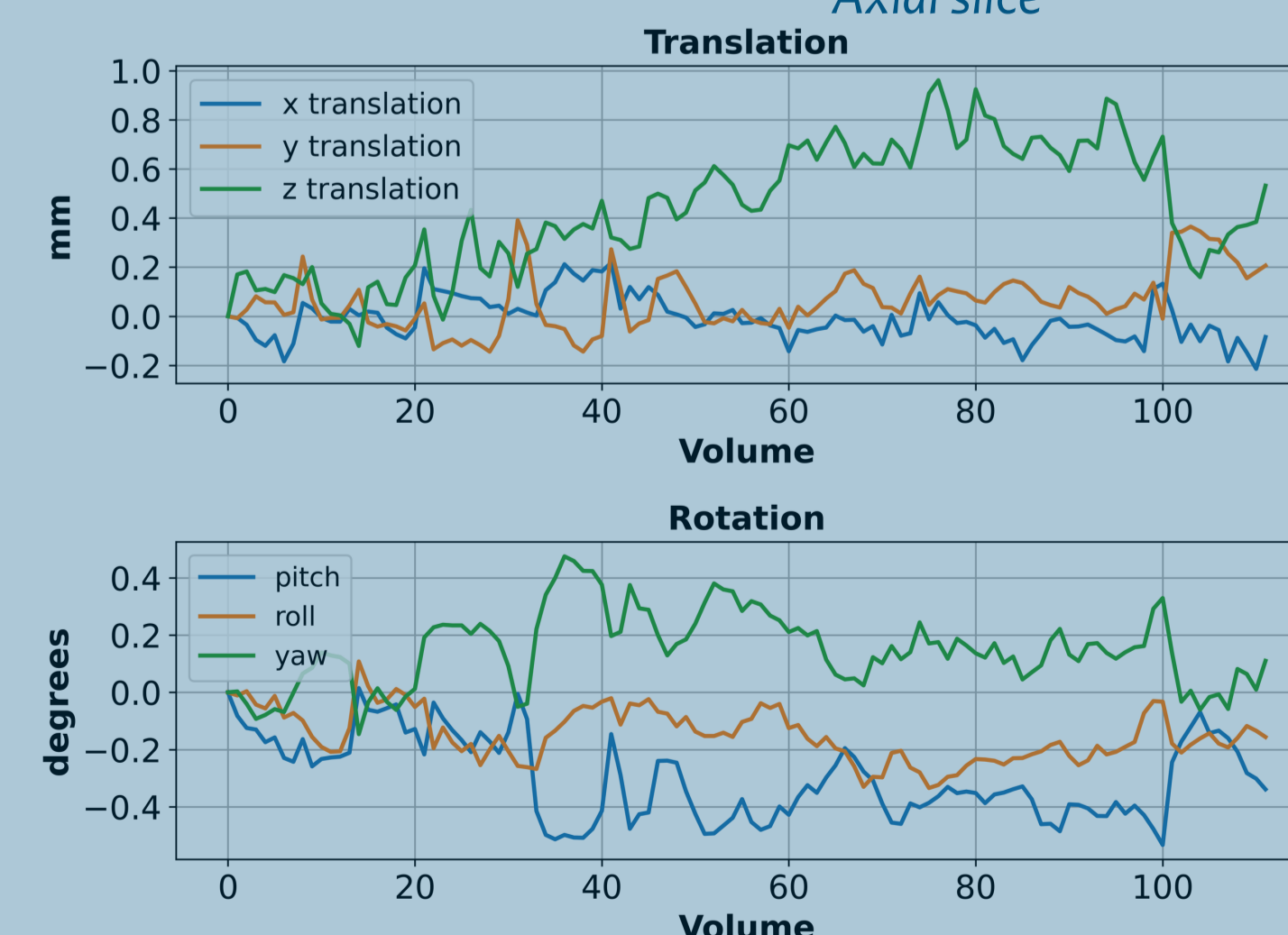
The activation overlay is thresholded at  $p < 0.001$  uncorrected



Coronal slice



Axial slice

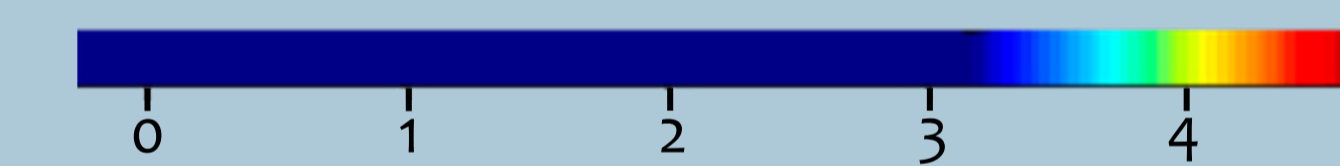


Motion parameters representing the amount of motion in each of the six directions for each volume acquired.

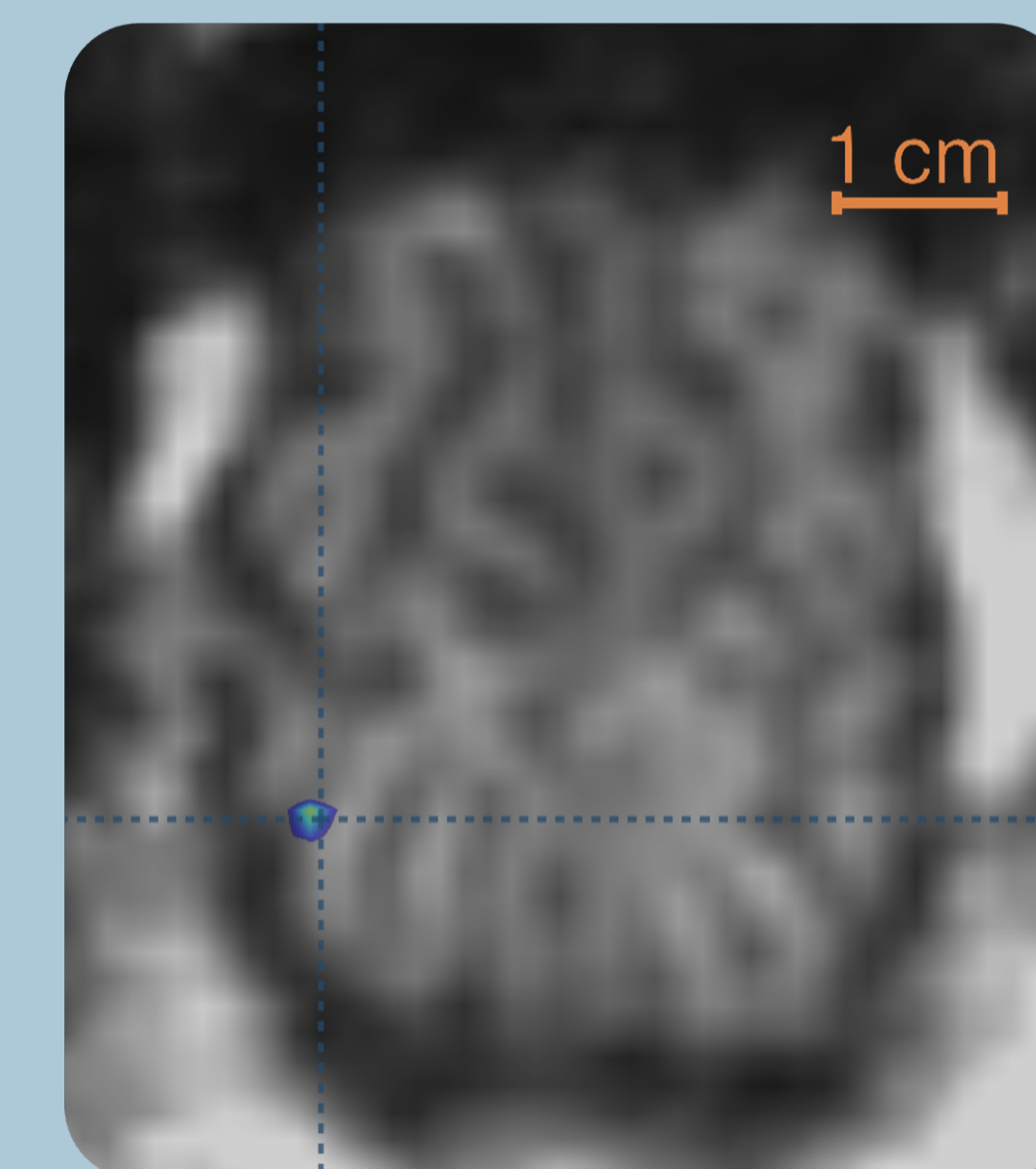
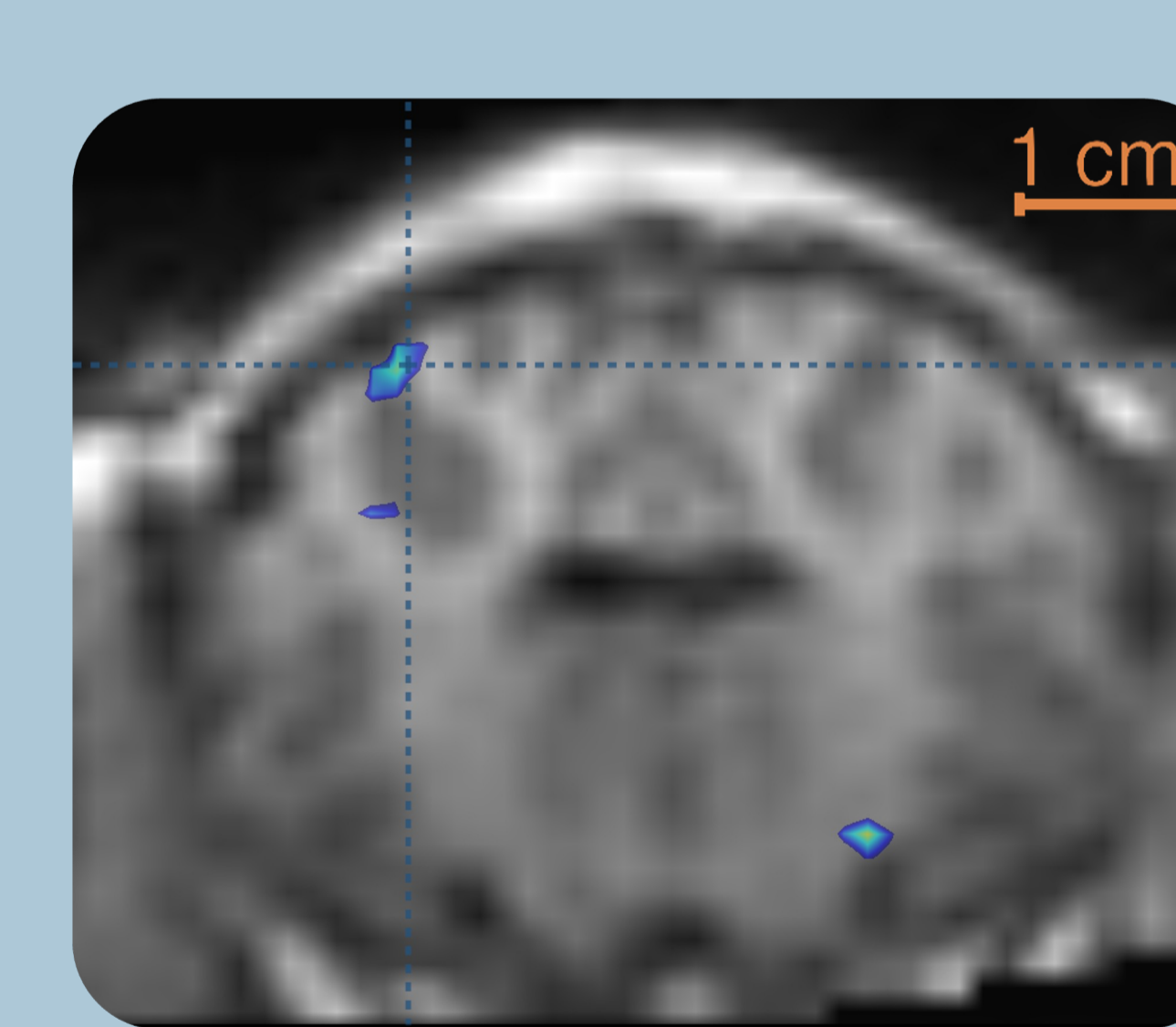
	Robin	Brook	Maggie	Ted	Léonard	Tony	Lily
LOCA (~30 sec)	✓	✓	✓	✓	✓	✓	✓
Run 1 (4 min 04)	✓	✓	✓	✓	✓	✓	
Fieldmap (1 min 57)	✓	✓	✓	✓	✓	✓	
T1 (2 min 36)	✓	✓	✓			✓	
Run 2 (4 min 04)	✓						

Progress in MRI data collection during a single session

BROOK



The activation overlay is thresholded at  $p < 0.001$  uncorrected



Motion parameters representing the amount of motion in each of the six directions for each volume acquired.